Brief Description of the Sequences

SEQ ID NO. 1 is a sense primer or amplification of FIV *gag* that can be used according to the present invention.

SEQ ID NO. 2 is a antisense primer or amplification of FIV *gag* that can be used according to the present invention.

Detailed Description of the Invention

The subject invention concerns materials and methods for detecting, preventing and treating infection by FIV in humans and other non-feline animals susceptible to infection by FIV. The present invention is based on the surprising discovery that FIV can be transmitted from cats to humans and can infect human cells *in vivo*. Human subjects have been identified that are FIV positive and appear to have been infected through contact with their pet cats. Infection of humans by FIV has been demonstrated by confirmation of the presence of FIV nucleotide sequences in human cells using polymerase chain reaction (PCR) and by Western blot detection of FIV proteins expressed in human cells. Sequence analysis confirms that the subject is infected with FIV. Both of the human subjects infected with FIV identified thus far are currently clinically and immunologically asymptomatic. It has also been demonstrated that antibodies to FIV cross-react with HIV proteins. In addition, antibodies from FIV vaccinated animals can neutralize HIV-1 virus. The subject invention also concerns materials and methods for preventing and treating infection by HIV in humans.

One aspect of the subject invention concerns methods for detecting FIV infection of human cells. One method of the present invention comprises detecting the presence of antibodies that bind to an FIV protein or peptide, or nucleotide sequences of FIV. FIV diagnostic tests of the invention include ELISA, Western blot, and PCR tests. Current commercially available HIV antibody tests cross react with FIV proteins and, therefore, can give "false positive" results in subjects which are not infected with HIV but which are infected with FIV. Thus, FIV diagnostic tests for humans are needed in facilities doing HIV testing, such as hospitals and blood banks, in order to screen false positives and indeterminant results

The present invention also concerns materials and methods for preventing FIV infection in humans and other animals. Specifically contemplated are methods and vaccine compositions which can be administered to human subjects and other susceptible host animals which will prevent infection by FIV. In one embodiment, an amount of an FIV immunogen effective to induce an immune response is administered to the human or animal. FIV immunogens that can be used include, for example, synthetic FIV peptide, natural or recombinant FIV protein or a fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or a fragment thereof and an HIV protein (such as NeF protein) or a fragment thereof, inactivated or attenuated whole FIV viral isolate, FIV viral fragment, inactivated cells infected with FIV, and compositions comprising FIV and HIV proteins or fragments thereof. In a preferred embodiment, the FIV immunogen comprises an epitope of an FIV protein, such as core gag protein or envelope protein, that is evolutionarily conserved between FIV and HIV. Persons that have higher exposure to cats, such as veterinarians, scientists that use cats for research purposes, cat breeders, etc., would be candidates for receiving a vaccine treatment. Other animals which can be treated according to methods of present invention include dogs, horses, and captive non-domesticated animals such as those found in zoos and circuses, including tigers and lions.

The subject invention also concerns materials and methods for treating persons and other animals that are infected with FIV. In one embodiment, an effective amount of a composition which can induce an immune response against FIV is administered to a person or animal in need of such treatment. In another embodiment, one or more antiretroviral drugs can be administered to the person or animal. Antiretroviral drugs which can be used in the present invention include, but are not limited to, nucleoside analogs, such as azidothymidine (AZT) and lamivudine (3TC), non-nucleoside inhibitors of retroviral reverse transcriptase, and retroviral protease inhibitors. Published international patent application WO 99/60988 describes the use of a combination of AZT, 3TC, and a retroviral protease inhibitor to treat

obtained from current HIV tests. Methods for detecting and diagnosing FIV are known in the art and can be readily incorporated into assays for the testing of biological samples from humans for HIV infection. U.S. Patent Nos. 5,037,753, 5,118,602, 5,275,813, 5,510,106, and 5,565,319 describe assays and compositions for detecting FIV. Materials and methods for detecting and diagnosing HIV are disclosed in U.S. Patent Nos. 4,708,818, 5,055,391, 5,108,891, 5,135,684, and 5,922,533. Diagnostic HIV assays are also commercially available from Bio-Rad Laboratories, Hercules, CA. Methods of testing biological samples from humans for FIV infection only (and not HIV) are also contemplated by the present invention. Methods for FIV detection include PCR assaying for proviral FIV nucleotide sequences, RT-PCR assaying for FIV RNA nucleotide sequences, oligonucleotide probe assays (including Real-time PCR), and antibody-based assays. Antibody-based assays include, for example, methods to detect the presence of antibodies to FIV, such as ELISA and Western blots, and methods to detect the presence and/or expression of FIV proteins in human biological samples. In one embodiment, a biological sample from a human that is being assayed for the presence of antibodies to HIV or HIV sequences is assayed for the presence of antibodies to FIV or FIV sequences.

The present invention concerns materials and methods for inducing an immune response to FIV in a human or non-feline animal that is susceptible to infection by FIV. The present invention also concerns materials and methods for inducing an immune response to HIV in a human. In one embodiment, an amount of an FIV immunogen effective to induce an immune response is administered to the human or animal. FIV immunogens that can be used include, for example, synthetic FIV peptide, natural or recombinant FIV protein or a fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or a fragment thereof and an HIV protein (such as NeF protein) or a fragment thereof, inactivated or attenuated whole FIV viral isolate, FIV viral fragment, inactivated cells infected with FIV, and compositions comprising FIV and HIV proteins or fragments thereof.

μg of UV-inactivated virus or cell lysate directly on the immunoblot strips with the serum for 2 hr and the immunoblots were developed as before. FIV-infected (FIV_{Shi}-infected FeT-J and FIV_{Bang}-infected FeT-J cell combination), HIV-infected (HIV-1_{UCDI} infected HuT-78 and HIV-1_{LAV} infected H9 cell combination) and uninfected (FeT-J alone or HuT-78/H9 combination) cells were inactivated by 0.6% paraformaldehyde. HIV-infected cells were also UV-inactivated before paraformaldehyde treatment. IgG levels of the cell-absorbed and unabsorbed mock sera were determined by commercial feline IgG radial-immunodiffusion assay (Bethyl Laboratory, Montgomery, Texas).

Cellular Immune Response: Virus-specific cellular immune responses of PBMC from vaccinated cats were determined by measuring the amount of interferon-γ produced in response to 10 μg/ml of recombinant FIV p24, HIV-1_{BRU} p24, and HIV-1_{IIIB} gp160 using the method previously described (Pu *et al.*, 1999). In addition, cells stimulated with uninfected cell lysate (20 μg/ml), SEA (0.2 μg/ml, positive control), media diluent (negative control), and purified whole FIV_{Pet} and FIV_{Shi} (20 μg/ml) were also included as additional controls.

Antibodies to FIV were developed in specific pathogen free (SPF) cats by either active infection with FIV strains or immunization with inactivated FIV vaccines. Sera from 41 FIV-infected cats at different time post-FIV inoculation were evaluated on BioRad HIV-1_{UCDI} and Cambridge Biotech HIV-1_{IIIB} immunoblots (Table 1, Figure 6A). Overall, 18 of 41 (44%) infected cats had antibodies to HIV-1 core capsid p24, matrix p18, Gag p55, intergrase p32, transmembrane envelope gp41, surface envelope gp120 or precursor envelope gp160 (Table 1, Figure 6A) with greatest reactivity to p24. Three of 10 cats infected with FIV_{Pet} (subtype A), 7 of 11 cats infected with FIV_{UK8} (subtype A), 5 of 11 cats infected with FIV_{Bang} (subtype A_{gag}/B_{env}), and 3 of 9 cats infected with FIV_{Shi} (subtype D) had cross-reactive antibodies to HIV-1. The majority of the cats (64%) infected with FIV_{UK8} developed cross-reactive antibodies to HIV-1, while only three cats (30%) infected with FIV_{Pet} developed cross-reactive antibodies to HIV-1. Both of these strains are subtype A FIV strains. Hence, strain specific cross-reactivity to HIV-1 may exist.

current HIV-1 antibody diagnostic tests that are used commercially to screen persons for infection with HIV.

Brief Summary of the Invention

The subject invention concerns materials and methods for detecting, preventing and treating retroviral infections in humans and other non-feline animals susceptible to infection by retrovirus. It has been discovered that FIV can be transmitted from cats to humans and that the FIV can infect human cells *in vivo*. Persons infected with FIV produce an immune response against the virus, including the production of antibodies to FIV. It has also been discovered that antibodies generated by a person infected with FIV cross-react with HIV antigens. Thus, the methods and compositions of the subject invention can be used to detect, prevent and treat FIV infection in humans and other non-feline animals that are susceptible to FIV infection. The present invention includes materials and methods for diagnosing whether a person is infected with FIV or HIV. The methods and compositions of the invention can also be used to prevent and treat infection by HIV in humans.

Brief Description of the Drawings

Figures 1A-1D show FIV Western blot analysis of subjects #FH1 and #FH2. FIV_{Shi} (D) and FIV_{Bang} (B) Western blots (Figures 1A-1C) were reacted with sera from subjects #FH1, #FH2, and #FH5 (control individual with minimum cat exposure) for 20 hours. Experimentally FIV-infected cat (Cat +) was used as the source of strongly reactive control serum and uninfected SPF cat (Cat -) was used as the source of non-reactive control serum. Key bands are highlighted with an arrowhead on the left. Figure 1D: Virus neutralizing antibodies to FIV and HIV were detected in cultures. a Western blot of human sera on FIV_{Shi}.

Figures 2A and 2B show alignment of gag sequences of cat #FC1 and subject #FH1. Figure 2A shows alignment of gag nucleotide sequences. Figure 2B shows alignment of gag amino acid sequences. Gag sequences of the nine clones isolated from cat #FC1 and subject #FH1 are shown in comparison to the consensus sequence of cat #FC1 (top sequence).

To date, there have been no reported cases of retroviral zoonosis between domestic cats and humans (Pedersen *et al.*, 1987; Yamamoto *et al.*, 1989; Yamamoto *et al.*, 1988; Butera *et al.*, 2000; CDC Report: HIV and Retrovirology). No cases of feline leukemia virus (FeLV), feline foamy virus (FeFV), and feline immunodeficiency virus (FIV) infections of humans have been reported, even in populations at high risk for viral exposure, such as veterinarians, animal caretakers, and scientists from feline retroviral laboratories (Yamamoto *et al.*, 1989; Yamamoto *et al.*, 1988; Butera *et al.*, 2000). Human patients with leukemia and chronic fatigue syndrome selected for their disease association also tested negative for FeLV (Butera *et al.*, 2000). However, many of the assays used in these studies were based on less sensitive antigen and antibody tests (Butera *et al.*, 2000). In few of these studies, a more sensitive FeLV PCR (polymerase chain reaction) system for proviral DNA and a sensitive Western blot analysis for FIV antibodies were performed but their findings also supported previous reports of the lack of feline retroviral zoonosis (Yamamoto *et al.*, 1988; Butera *et al.*, 2000). *In vitro* studies have shown that all three of these feline retroviruses are capable of infecting primary human cells and human cell (Butera *et al.*, 2000; Sarma *et al.*, 1970; Azocar *et al.*, 1979; Jarrett *et al.*, 1973). Recent studies have also demonstrated that FIV infects human cells *in vitro* via the CXCR4 receptor, which has been shown to be a coreceptor for HIV-1 (Willett *et al.*, 1997a; Willett *et al.*, 1997b; Poeschla *et al.*, 1998; Richardson *et al.*, 1999; Johnston *et al.*, 1999b). It has been reported that FIV vector sequences which included FIV *rev-RRE* and *gag* are more efficient in infecting human cells than those without FIV *gag* (Johnston *et al.*, 1999).

Zoonotic infection of humans with SIV has been limited to individuals working with SIV or SIV-infected laboratory animals (Khabbaz *et al.*, 1994; Khabbaz *et al.*, 1992). All of the SIV-infected individuals are clinically asymptomatic with one having transient infection while the other showed persistent infection.

Disclosed herein is the surprising discovery of zoonotic retroviral infection of humans *in vivo* with FIV. It has also been discovered that zoonotic FIV infection can complicate the